The Resistance of Organic Materials to Attack by Marine Bacteria at Low Temperatures

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The use of various organic materials in submarine cable structures has brought about an interest in the anticipated behavior of such materials in a sea water exposure. Organic materials have been exposed in the laboratory at atmospheric pressure to marine bacteria at both 20°C and 5°C under aerobic conditions and 20°C under anaerobic conditions. The utilization of the test materials by the bacteria has been determined by either measurements of oxygen consumption or hydrogen sulfide production.

None of the polyethylenes, synthetic fibers, or other thermoplastics such as polytetrafluoroethylene, polycarbonate, or polyamide was utilized by either aerobic or anaerobic microorganisms. All the elastomers tested were utilized by aerobic bacteria, but a majority of them were resistant to attack by anaerobic bacteria. Jute fibers served as a carbon source for both aerobic and anaerobic bacteria. In general, the poly(vinyl chloride) materials plasticized with an external plasticizer served as a source of energy for aerobic microorganisms at both temperatures, and six of these were also utilized by anaerobic bacteria. It appears that the added external plasticizer was responsible for the attack, since further tests showed the pure resin and the internally plasticized poly(vinyl chloride) to be resistant to both types of bacteria.

Comparison of the data from 20°C and 5°C aerobic tests indicates greater microbial activity at the warmer temperature. Calculated rates of utilization of the susceptible materials showed that the elastomeric compounds were generally consumed ten times as fast when incubated at 20°C as at 5°C. The poly(vinyl chloride) compounds when compared similarly showed approximately a five-to-one ratio, while jute fibers were utilized twice as fast at 20°C than at 5°C.

I. INTRODUCTION

The interest of the Bell System in the extent of marine bacterial activity at great ocean depths stems from the knowledge that certain organic materials are susceptible to attack by such organisms. For example, studies made by ZoBell and Beckwith[1] on rubber products and by ZoBell[2] on hydrocarbons have indicated that marine bacteria are capable of utilizing these materials. Therefore, one phase of a biological testing program (see Fig. 1) initiated by the Laboratories in 1954 has been designed to determine the resistance of organic materials that were possibly applicable in ocean cable construction to attack by both aerobic and anaerobic marine bacteria. The first data collected from these laboratory tests have been reported earlier by Snoke,[3] and showed that in many instances specific organic materials were capable of being utilized by marine bacteria at 20°C. Attack was predominately by aerobes, little attack having occurred due to sulfate-reducing anaerobes.

The 20°C temperature is representative of certain shallow water environments and is in the range which supports high microbial activity. Ocean bottom temperatures at depths greater than 1000 meters range from 5° to −1.5°C.[4] The present work, concerned with deep water temperatures, involves the testing of the resistance of the materials to aerobic bacteria at an incubation temperature of 5°C and the comparison of the results with those at 20°C. It was assumed initially that the rate of activity would be somewhat lower at 5°C, and since, in most cases, there had been little attack by anaerobic organisms at 20°C, no 5°C anaerobic tests were conducted.

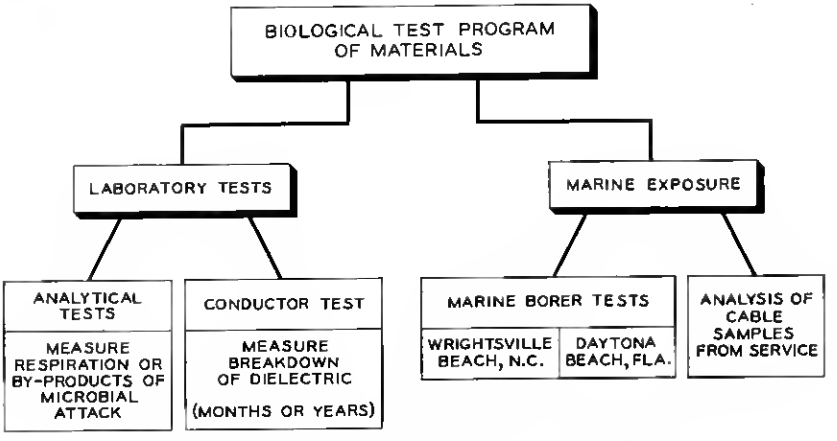

Fig. 1 — Outline of marine biological test program.

II. BACKGROUND

Marine bacteria are found in varying quantities over the entire ocean, with the greatest concentration being in coastal waters and in the region of the sediment-water interface. In spite of their minute size (generally less than one micron in length and one micron in diameter) they are so plentiful that the estimated mass of bacteria in the oceans has been placed at ten million tons.[5] The concentration at a particular time at any location reflects the existing balance between productive and destructive factors. The numbers of bacteria are predominately due to the amount of organic matter present, since this provides the food source for the bacteria. An example of the influence of the quantity of organic matter on the bacterial population can be seen in the studies made by Hesse on water from the junction of the Atlantic Ocean and the colder Arctic Ocean.[6] The change in temperature encountered here kills large quantities of marine organisms, thus providing an increase in the organic food supply, and has resulted in a great increase in the number of bacteria.

Other environmental factors may also have some influence on the bacterial population through effects on the supply of organic matter. It has been noted that a direct relationship exists between the bacteria and the diatoms, unicellular marine plants. These plants are capable of synthesizing organic matter from the simple chemical substances produced during the bacterial decomposition of extraneous organic material. Therefore, in areas of abundant diatoms there may also be an abnormally high number of marine bacteria. One area with a characteristically high population of marine bacteria is the mud-water interface. The population increase here is due to the rich food supply caused by constantly sinking marine plant and animal remains. Of this utilizable organic matter, Waksman and Carey found that approximately 60 per cent consumed by the bacteria is oxidized to carbon dioxide and water, and that the remainder is used to build new bacterial cells.[7]

Marine bacteria have been found in bottom deposits at depths of 10,280 meters below sea level, where the hydrostatic pressure is approximately 1000 atmospheres.[8] Cattell[9] indicates that single-cell microorganisms can tolerate pressures up to 6000 atmospheres. Several species of bacteria studied by Sanboru[10] were active at a temperature of −5°C, which is slightly lower than typical bottom temperatures in the deep ocean. However, in spite of the ability of marine bacteria to adapt to different conditions, there are several factors that do restrict their number in the ocean. First, the amount of organic matter in the open sea is limited, and of this supply a significant part cannot be utilized by the

bacteria. Second, bacteria are a major part of the food supply consumed by protozoa and other small marine animals. Third, as the rate of bacterial multiplication increases, a toxic substance injurious to their development may begin to form and thus partly inhibit further growth.

Temperature seems to influence the bacterial environment, mainly as a factor controlling the extent of activity, but with little effect on gross population figures. Studies have indicated that bacteria found in the coldest waters multiply more slowly but live longer. ZoBell[4] has also found that this relationship holds true for sea water stored in glass receptacles at incubation temperatures ranging from 0° to 25°C. Temperature may restrict the number of species of bacteria present in a given location, but the only time it becomes a factor influencing the total bacterial population is prior to the establishment of an equilibrium within the system.

This brief outline of marine bacteria illustrates the complexities facing the experimenter who is interested in trying to simulate their ecology. Since there are so many variables, any laboratory tests will necessarily be limited by the particular set of conditions satisfied during incubation of the test bottles. The tests reported here were conducted under laboratory conditions using aged and filtered sea water, laboratory-incubated enrichment cultures, and small test samples. Due to the great number of samples, all could not be tested at one time. Also, any chemical changes which might occur in the materials due to prolonged exposure were not reflected. The data collected in these tests do provide immediate information on the relative performances of the materials under the test conditions, and can be used as a gauge in judging materials until natural exposure data are available.

The first results from early inspections of samples exposed directly in the ocean were reported by Snoke;[3] more recent data will be made available in a future paper.

III. DESCRIPTION OF LABORATORY TESTS

The two types of laboratory tests conducted are designed to test the resistance of the various organic materials to both aerobic and sulfate-reducing bacteria. The latter test, since sulfate-reducing bacteria are ubiquitous, gives an indication of the extent of anaerobic attack. In both cases, the procedure consists in exposing the test materials to bacteria which must utilize the material as a carbon source if they are to live.

Whenever possible, the materials to be tested are obtained in the form of film about 4 mils thick. Each bottle contains enough sample to expose a surface area of 12.9 square centimeters, which, in the case of film, is a 1-inch-square sample. The elastomeric materials are approximately

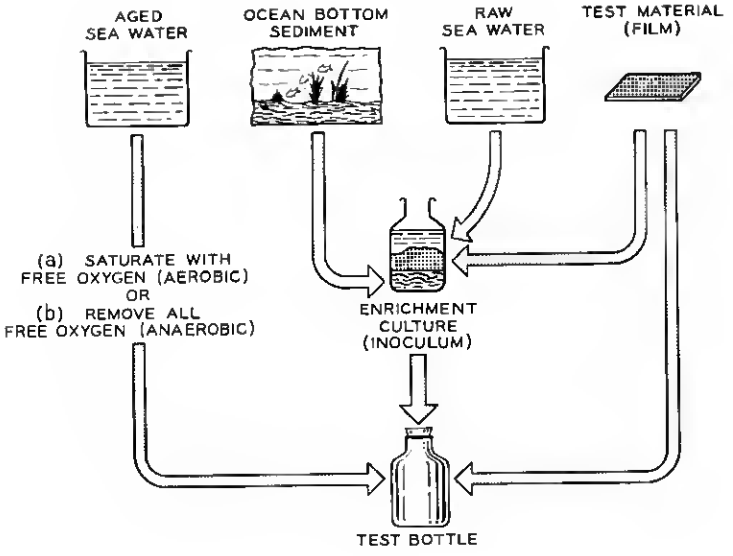

Fig. 2 — Flow chart of laboratory bacteriological test.

75 mils thick and are therefore cut in $\frac{15}{16}$-inch-square samples. It is difficult to measure the surface area of granular samples and, as a result a selected mass, namely 0.05 gram, is used for all such samples or for jute fiber. The sample size for the other fibers, namely acrylonitrile-vinyl chloride copolymer and 6-6 polyamide (Source Ib)*, was determined by an approximation of the desired 12.9 square centimeter surface area.

3.1 *Aerobic Bacteria*

The biochemical oxygen demand (BOD) type of test measures the amount of dissolved oxygen consumed by aerobic bacteria during an eight-week incubation of the organic materials in aged and inoculated sea water. Since the test samples supply the only source of carbon available to the bacteria, the rate of oxygen consumption, which is a measure of the respiratory process of the living system, becomes a direct measure of the utilization of the material. The flow chart representing the steps involved in the test preparation is shown in Fig. 2.

At least eight weeks prior to running the test, sea water and marine sediment are collected from an area on the Atlantic coast. Some of the material to be tested is added to a portion of the collected sea water and sediment, and the mixture is inoculated from pooled marine en-

* Indicates source of supply.

richment cultures obtained through the courtesy of C. E. ZoBell of Scripps Institute of Oceanography. These latter were prepared using a wide range of organic materials including elastomers, carbohydrates, coal products, and other aromatic compounds. The enrichment cultures made up for each test sample are incubated at 25°C for at least six weeks prior to testing. The purpose of preparing separate cultures is to enable bacteria capable of utilizing the materials to develop preferentially under ideal conditions. In the meantime, the remainder of the raw sea water is filtered and aged in the dark until the utilizable organic content is about one part per million or less.

When these preliminary procedures have been carried out, the aged sea water is placed in a carboy and aerated with oxygen for 16 hours. Prior to filling the test bottles, a combined enrichment culture containing a few drops from each of the incubated cultures of the sample group being tested is added to the aerated sea water in a concentration of 1 milliliter per 10 liters of sea water. This concentration of inoculum provides maximum microbial activity with the least amount of added organic material. A slight positive oxygen pressure is placed on the water in the carboy until after the test bottles are filled, thus assuring oxygen saturation throughout the period preceding incubation.

Ten replicates of each sample are incubated in 60-milliliter glass-stoppered bottles in a constant-temperature water bath. Normally, two replicate samples are sacrificed for measuring the dissolved oxygen of the water following 0, 1, 2, 4, and 8 weeks of incubation. However, when a high rate of attack is anticipated the incubation intervals are shortened in order to obtain significant titration data before all the available oxygen has been utilized by the bacteria. Usually six materials are included in each test run with one set of control bottles. The 10 control bottles included contain inoculated sea water and are prepared exactly the same as the test bottles except for the absence of test material.

The dissolved oxygen content of the test bottles before incubation and following the different incubation periods is measured using a modified Winkler method. The basic Winkler procedure consists of the three steps shown in Fig. 3: (1) oxidation of manganous hydroxide in a highly alkaline solution, (2) acidification of the product in the presence of an iodide, and (3) volumetric titration of the liberated iodine with a standard thiosulfate solution using a starch indicator.

The oxygen consumed is calculated in parts per million from the results of the titrations of the sea water prior to incubation and following the longest period of incubation. The difference between these two values over and above any loss of oxygen in the control samples constitutes the biochemical oxygen demand (BOD) with the test material.

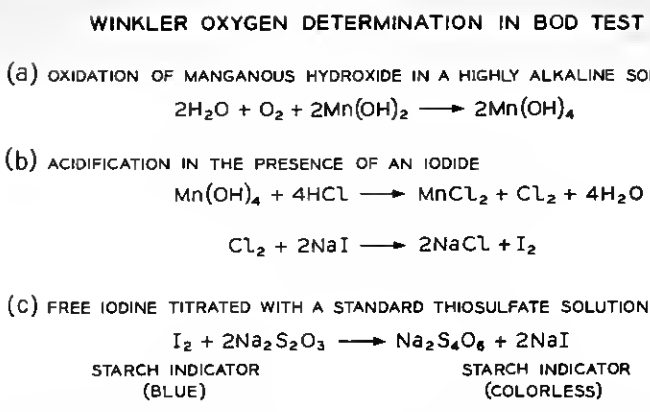

Fig. 3 — Reactions involved in laboratory marine analytical tests.

3.2 *Anaerobic Bacteria*

In the anaerobic test procedure the volume of hydrogen sulfide produced as a by-product during the utilization of the organic materials by anaerobic sulfate-reducing bacteria is measured as an indication of anaerobic attack.

Except for the handling of the aged sea water, the preparations for the anaerobic test are quite similar to those for the BOD test (see Fig. 2). In this test the sea water is boiled vigorously for 10 minutes and siphoned immediately into a carboy. A slight positive nitrogen pressure is placed on the carboy until the water is cooled enough to add the inoculum. Additional nitrogen pressure is kept on the carboy while the bottles are filled in order to prevent free exchange of oxygen with the water. Since the anaerobes function much more slowly than the aerobic bacteria the incubation periods selected are 0, 4, 8, 12, and 16 weeks in the water bath at 20°C.

Following incubation the hydrogen sulfide dissolved in the sea water is titrated with an iodine solution using starch as an indicator (see Fig. 3). Hydrogen sulfide content calculated over and above that found in the inoculated controls is considered a measure of anaerobic attack of the material in test.

IV. MATERIALS TESTED

The individual compounds which have been tested against aerobic and anaerobic organisms are listed in Table I. They included elastomers, poly(vinyl chloride), polyethylene, polypropylene, other thermoplastics, natural and synthetic fibers, and casting compounds.

4.1 *Elastomers*

With the exception of silicone rubber, all rubber samples were compounded at Bell Telephone Laboratories, steam cured, and supplied in sheet form approximately 75 mils thick. Although a few materials were submitted as both jackets and insulation the composition of the two varied considerably in some cases, and the two compounds behaved quite differently in test.

According to ZoBell and Beckwith,[1] even a highly purified nonvulcanized rubber sample can be utilized by marine bacteria. The presence of numerous waxes, oils, soaps, stearic acid, and phenolic compounds used in the compounding of the elastomers tends to increase the susceptibility of the rubber compounds to microbial attack. To date, no work has been done to determine which components in the elastomers were primarily responsible for the extent of attack experienced in the laboratory.

4.2 *Poly(Vinyl Chloride)*

All poly(vinyl chloride) samples submitted in sheet form were approximately 2 to 5 mils thick. All of the known plasticizers in the externally plasticized poly(vinyl chloride) samples contained typical organo-metallic-type stabilizers (such as barium, cadmium, and lead), fatty acid lubricants in low concentrations, and, in some cases, small quantities of inorganic fillers.

The unplasticized, rigid poly(vinyl chloride), although not containing a specific external plasticizer, was compounded with a significant amount of fatty acid lubricant and is therefore compared throughout the text with the externally plasticized compounds. This compound contained approximately eight to ten times as much fatty acid as was used in compounding the two internally plasticized acrylate copolymers designated semiflexible copolymers A and B. The rigid poly(vinyl chloride) should not be confused with poly(vinyl chloride) resin also discussed here, which was a pure poly(vinyl chloride) powder containing no added plasticizer or lubricant. Polyester A, C, and E plasticizers were fatty

TABLE I—MATERIALS TESTED AGAINST AEROBIC AND ANAEROBIC MARINE BACTERIA

Material	Chemical Department Designation
Elastomers	
GR-S jacket	BTL 54-14
Natural rubber jacket	BTL 54-23
Butyl rubber jacket	BTL 54-19
Neoprene red lead jacket (with clay)	BTL 54-9
Neoprene red lead jacket (without clay)	BTL 54-1
Natural rubber insulation	BTL 54-24
GR-S insulation	BTL 54-22
Silicone rubber	BTL 53-83
GR-A jacket	BTL 54-18
Neoprene (ZnO, MgO jacket)	BTL 54-164
Poly(vinyl chloride) — plasticizer used	
Polyester E (BTL 46-55)	P5503115
Polyester A	BTL 24-54
Di-2-ethylhexyl phthalate (DOP), Source 1 — sheet	P5606412
None — rigid	P5503087 and P5402049
Tri-2-ethylhexyl phosphate (TOP)	P5502081
Nitrile rubber/polyester C	P5502074
Nitrile rubber	P5502076
Tricresyl phosphate (TCP) — sheet	P5311580
Di-2-ethylhexyl phthalate (DOP), Source 3	P5511691
Proprietary compound	P5511690
Di-2-ethylhexyl phthalate (DOP), Source 2	P5502082
Tricresyl phosphate (TCP) — granular	BTL 529-53
Di-2-ethylhexyl phthalate (DOP), Source 1 — granular	BTL 23-54
None (semiflexible copolymer A)	P5502078
None (semiflexible copolymer B)	P5502077
None (PVC resin)	P5510645
Polyethylene	
0.2 melt index + antioxidant — sheet	P5308390
0.2 melt index + antioxidant + 5% butyl rubber-sheet	P5602065 and P5403122
0.2 melt index (Source B) — sheet	P5312587
2.0 melt index (ASTM D1238) — granular	P5310466
0.2 melt index (Source A) — granular	P5304156
0.2 melt index (Source B) — granular	P5312587
0.2 melt index + antioxidant — granular	P5308390
0.2 melt index + antioxidant + 5% butyl rubber — granular	P5308396
0.7 melt index (high density) natural + antioxidant — sheet	P5512792
0.7 melt index (high density) + carbon black + antioxidant — sheet	P5503133
Polypropylene	
Polypropylene + antioxidant A	
Polypropylene + antioxidant B	

TABLE I—CONTINUED

Material	Chemical Department Designation
Other thermoplastics	
Cellulose acetate	
Cellulose triacetate	
6-6 polyamide (Source Ia)	
6 polyamide (Source II)	
6-10 polyamide (Source III)	
Polycarbonate	
Polyether	
Polyethylene terephthalate	
Polytetrafluoroethylene	
Tritheue film	
Fibers	
Acrylonitrile-vinyl chloride copolymer	
6-6 polyamide (Source Ib)	
Casting compounds	
Epoxide	LRM 392, L2 (sheet)
Styrene-polyester, silica filled	LRM 326, L1 (sheet)

acid compounds with polyester C having been compounded with nitrile rubber for use as a test material.

Two of the compounds containing di-2-ethylhexyl phthalate (DOP) as the external plasticizer are labeled Source 1 and Source 2, and may be differentiated by the Shore A hardness test.* Source 1 had a Shore A figure of 88, indicating that it contained more plasticizer than Source 2, which had a Shore A figure of 62. The concentration of di-2-ethylhexyl phthalate in Source 3 is not known.

4.3 *Polyethylene*

The 0.2 melt index (Source A) and 2.0 melt index samples were prepared only in granular form. Data for some of the other polyethylenes were tabulated for both sheet and granular forms, but comparisons were only made when both series of tests involved the use of a similar form of material.

4.4 *Polypropylene*

Both polypropylene samples were submitted in sheet form about 15 mils thick. One sample contained antioxidant A, which included a phenolic compound, and the other contained an unknown antioxidant (B).

* See ASTM D-1706-59 T.

4.5 *Other Thermoplastics*

Most of the other thermoplastics tested were supplied as films between 1 and 5 mils thick. Of the polyamide samples tested, Source Ia is a 6-6 polyamide, Source II is a 6 polyamide, and Source III is a 6-10 polyamide. The cellulose acetate sample contained 54.5 per cent acetic acid, while the cellulose triacetate contained 61.5 per cent acetic acid.

4.6 *Fibers*

The jute used was an untreated roving. Acrylonitrile-vinyl chloride copolymer and 6-6 polyamide (Source Ib) were both supplied as yarn. Since both of these latter materials had been subjected to spinning oils during manufacture, they were exposed in test both as unwashed and washed samples. Petroleum ether was used as a wash medium.

4.7 *Casting Compounds*

The granulated epoxide compound was a casting resin set up through the use of an epoxy hardener. The sheet compound was a silica filled epoxy resin which had been pigmented with titanium oxide and hardened with an amine hardener. The resulting compound was quite brittle and about 13 mils thick.

The granulated styrene-polyester compound was also silica filled. The similar sheet material provided was filled with silica (39.7 parts) and glass fiber (5 parts) and pigmented white with TiO_2. This compound was approximately 10 mils thick and was very brittle.

V. TEST RESULTS

In discussing the results of this test program the word "attack" is used quite freely when describing the utilization of organic materials by the bacteria. The word "attack" is used here to indicate that a material can serve as a source of food for marine bacteria rather than to indicate that it suffers rapid visible deterioration. In natural exposure this "attacked" material might perform quite satisfactorily for many years; in fact, many similar materials have been used successfully in marine environments over the years. However, the accelerated test data are valuable in screening various materials as to their comparative expected performances. Such data are of value in the selection of materials for submarine applications until long-range tests have been in operation for a considerable length of time. Also, when the natural exposure data do become available they can be correlated with the laboratory figures to provide additional information.

Figs. 4 and 5 show the extent of attack of the elastomers and the externally plasticized poly(vinyl chloride) samples as functions of time. They are based on data collected from the Winkler titrations, which measure the dissolved oxygen content of the sea water after the various incubation intervals. Each individual point on the curve was calculated as follows:

$$\frac{A - B_x}{A} \times 100 - C = \text{per cent of oxygen consumed during particular incubation interval,}$$

where

A = dissolved oxygen (ppm) present in test bottles prior to incubation (average of two replicate samples),

B_x = dissolved oxygen (ppm) present in test bottles following the incubation period under consideration (average of two replicate samples),

C = per cent oxygen loss in the control or inoculated sea water bottles during the same incubation interval (x).

Whenever the available supply of dissolved oxygen was utilized entirely, no control factor (C) was used, and the result was reported as 100 per cent consumption. In general, the loss of oxygen in the control bottles during eight weeks' incubation at 20°C was between 10 and 15 per cent of the total dissolved oxygen present. In the tests run at an incubation temperature of 5°C, 5 to 10 per cent of the total oxygen was usually lost during the same interval.

In a few instances of low oxygen consumption the two-week titration figure showed more dissolved oxygen remaining than at the end of one week of incubation. Such anomalies may result from the fact that either (a) the control bottles showed more loss of oxygen than usual after two weeks of incubation or less than usual in the bottles titrated following one week, (b) the systems were not exact replicates, or (c) there was a small variation in the initial supply of oxygen. In such instances the higher oxygen consumption value was entered for both test periods to reflect the higher rate of attack.

To permit a ready comparison of the attack of different materials by aerobic bacteria, an "index of oxygen consumption" was calculated for each material. This index is the average daily consumption for the incubation period up to 100 per cent consumption or to the longest test interval (eight weeks), whichever is shorter. Thus the units of the index are properly "per cent oxygen consumed by the bacteria per day." It is

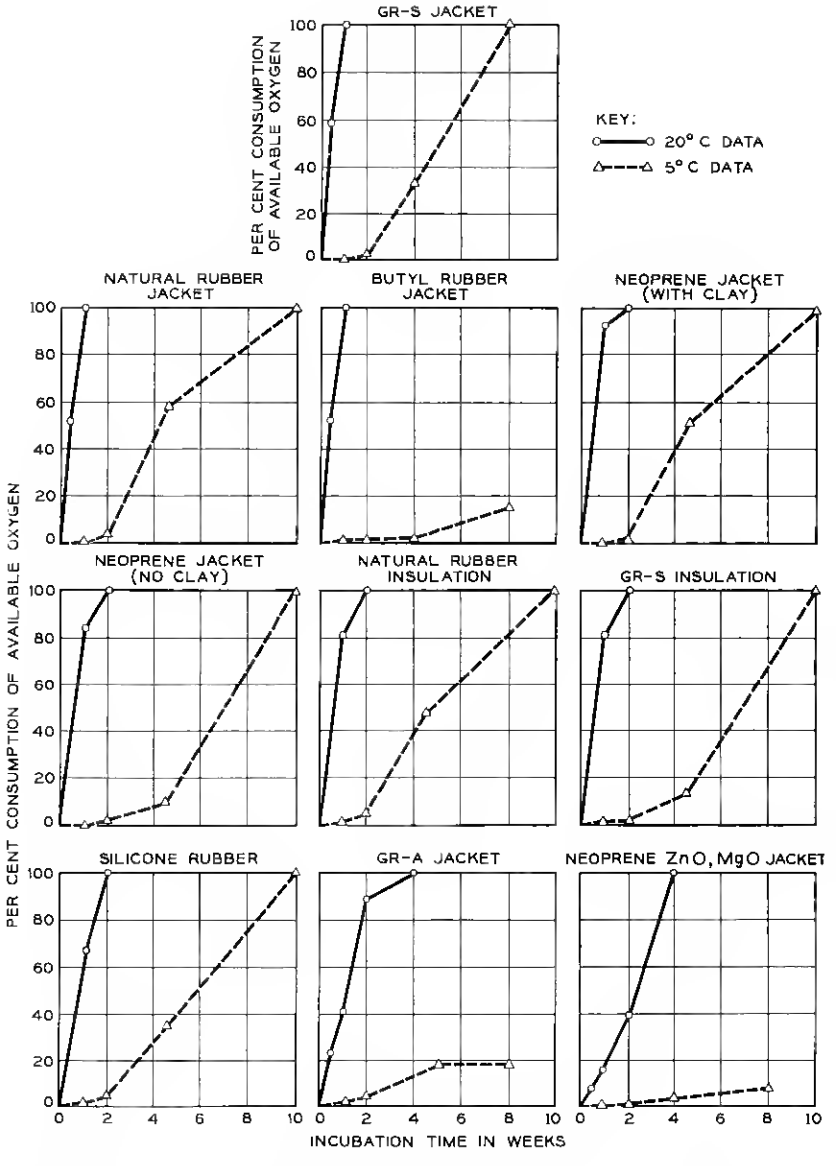

Fig. 4 — Individual biochemical oxygen demand (BOD) test results on elastomers.

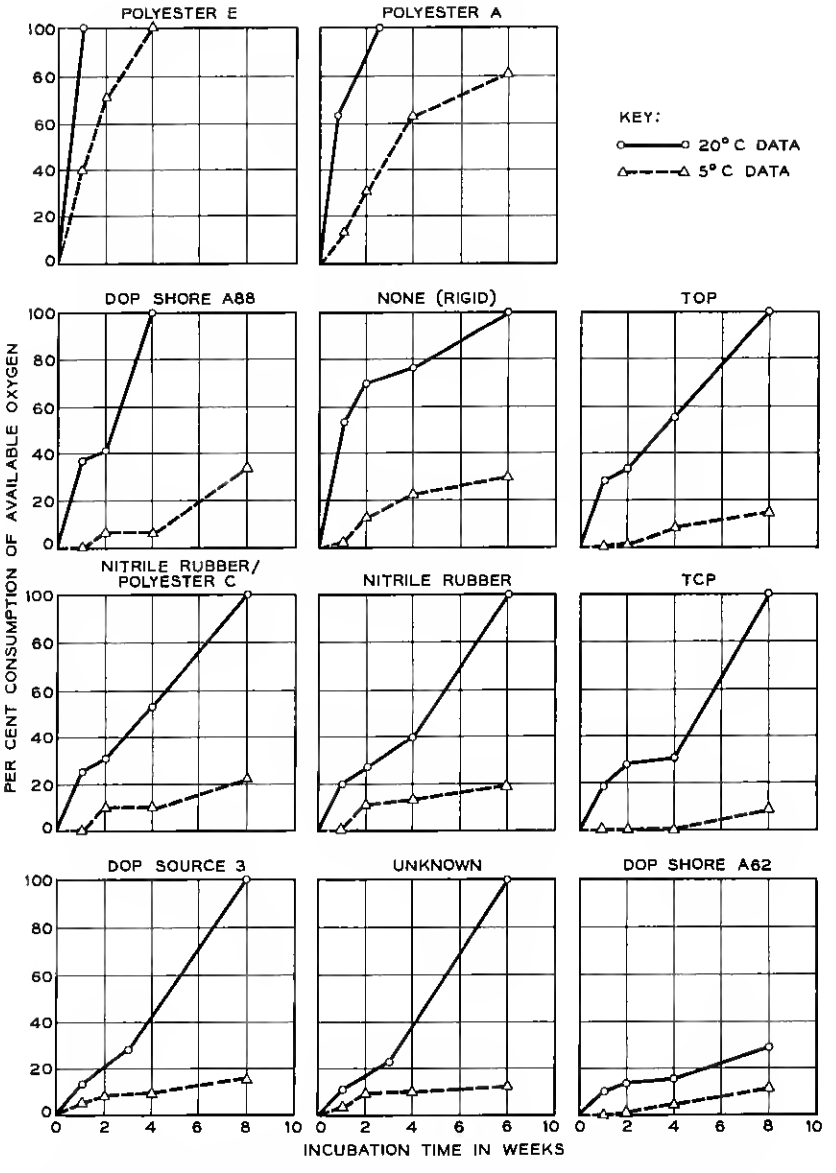

Fig. 5 — Individual biochemical oxygen demand (BOD) test results on poly-(vinyl chloride) plastics; samples are designated by plasticizer used.

preferable, however, to disregard the units in dealing with the index, since the consumption rate is seldom linear; hence the index cannot be used to calculate oxygen consumption reliably. Its value is in providing a single figure which qualitatively reflects relative rates of attack while the curves of Figs. 4 and 5 provide more detailed comparisons.

The indices of oxygen consumption figures for all test materials are listed in Table II. In the interpretation of these data, a material was arbitrarily judged a potential food source for the marine bacteria if the index of oxygen consumption exceeds 0.5 per cent per day in the 20°C tests. Such materials are indicated by asterisks. Also, if BOD test results showed a system to be capable of utilizing more than 0.1 per cent of the available oxygen per day in the 5°C tests, the quality of the performance of this material was questioned.

The comparison of the indices calculated for the individual materials at 5° and 20°C has been made by a general grouping of the materials according to their rates of utilization, and may be found in Table III.

A list of materials that were not attacked in aerobic or anaerobic tests may be found in Table IV.

VI. DISCUSSION OF TEST RESULTS

Curves drawn for all of the externally plasticized poly(vinyl chloride) samples and the elastomers (Figs. 4 and 5) suggest that there are three general patterns of oxygen consumption by the marine bacteria. Essentially these are as follows: *Type A* — oxygen consumption rate follows a linear pattern as a function of time; *Type B* — oxygen consumption rate increases as the length of incubation time increases; and *Type C* — oxygen consumption rate decreases as the length of incubation time increases. In all cases involving the BOD testing of elastomeric materials at 20°C the oxygen was consumed in a manner illustrated by the Type A slope, in which the rate of oxygen consumption appears to be a linear function dependent on the length of incubation time. However, in most of these tests the total available oxygen was utilized so quickly that very little titration data were collected, and it was rather difficult to generalize concerning the manner of attack of the materials. In many cases only two points could be considered, since the exact time at which 100 per cent of oxygen had been consumed was not known. When the same materials were incubated at 5° only neoprene (containing zinc oxide and magnesium oxide) jacket was attacked at a rate directly proportional to time. GR-A jacket was utilized more readily at the beginning of the test, and the remainder of the elastomers were utilized more easily as the incubation time increased.

TABLE II — COMPILATION OF DATA FROM 20°C AND 5°C BOD TESTS AND 20°C ANAEROBIC TEST

Material tested	Form of material tested	Individual 20° BOD test results — per cent available oxygen consumed				Individual 5° BOD test results — per cent available oxygen consumed				Index of oxygen consumption — per cent per day		Anaerobic test results — total H_2S produced
		1 week	2 weeks	4 weeks	8 weeks	1 week	2 weeks	4 weeks	8 weeks	20°C	5°C	16 weeks
Elastomers												
GR-S jacket	sheet	3 days 59.8	1 week 100			0	3.5	34.3	100	19.9*	1.2*	0
Natural rubber jacket	sheet	3 days 53.4	1 week 100			0	3.5	(32 days) 58.8	(70 days) 100	17.8*	1.8*	3.0*
Butyl rubber jacket	sheet	3 days 52.7	1 week 100			1.3	2.0	2.0	15.0	17.6*	0.3*	9.3*
Neoprene red lead jacket (with clay)	sheet	93.2	100			0	1.0	(32 days) 51.4	(70 days) 100†	13.3*	1.6*	0.1
Neoprene red lead jacket (without clay)	sheet	84.1	100			0.3	2.0	(32 days) 10.2	(70 days) 100	12.0*	1.4*	0
Natural rubber insulation	sheet	81.6	100			1.3	4.5	(32 days) 48.3	(70 days) 100†	11.7*	1.5*	1.7*
GR-S insulation	sheet	81.5	100			1.3	1.3	(32 days) 13.0	(70 days) 100	11.6*	1.4*	0.4
Silicone rubber	sheet	68.1	100			1.6	4.9	(32 days) 35.8	(70 days) 100†	9.7*	1.4*	0
GR-A jacket	sheet	41.3	89.0	100		1.0	4.1	18.0	18.0	6.4*	0.3*	0.1
Neoprene jacket (ZnO, MgO)	sheet	15.5	39.0	100		0	0.1	2.8	7.4	2.8*	0.1*	0.4

Poly(vinyl chloride)—designated by plasticizer												
Polyester E	sheet	100				39.7	71.7	100		14.3*	5.1*	82.3*
Polyester A	granular	(5 days) 62.8	(17 days) 100			13.3	31.7	61.5†	81.2	10.3*	2.2*	47.1*
Di-2-ethylhexyl phthalate (DOP) — Source 1	sheet	37.3	41.3	100		0	6.2	6.2	34.0	3.0*	0.6*	1.5*
Unplasticized (rigid)	granular	52.4	69.5	76.6	100	2.4	12.6	22.2	29.6	2.7*	0.5*	5.0*
Tri-2-ethylhexyl phosphate (TOP)	sheet	28.7	34.8	56.2	100	0.6	1.4	8.4	14.1	2.0*	0.3*	0.4
Nitrile rubber/polyester C	sheet	26.1	32.4	54.9	100	1.2	8.8	8.8	22.2	2.0*	0.4*	18.0*
Nitrile rubber	sheet	19.9	27.2	40.5	100†	0.7	11.6	13.2	18.6	1.8*	0.3*	10.9*
Tricresyl phosphate (TCP)	sheet	18.2	28.0	30.7	100†	0	0	0	9.5	1.8*	0.2*	0
Di-2-ethylhexyl phthalate (DOP) — Source 3	granular	13.9	(3 weeks) 28.8		100	6.2	8.3	9.3	15.0	1.4*	0.3*	0
Unknown	granular	10.4	(3 weeks) 22.2		100	2.7	9.3	9.3	12.8	1.1*	0.2*	0
Di-2-ethylhexyl phthalate (DOP) — Source 2	sheet	10.5	14.1	16.2	28.6	0	2.0	4.8	11.4	0.5*	0.2*	0
Tricresyl phosphate (TCP)	granular	14.4	(17 days) 45.7	64.7	100					2.3*		0
Di-2-ethylhexyl phthalate (DOP) — Source 1	granular	11.1	(17 days) 44.3	52.8	100					1.9*		0
None—copolymer A	sheet	2.1	4.0	5.4	5.4	0.3	0.3	0.3	0.4	0.1	0	0.4
None—copolymer B	sheet	0	0	0	0	0.8	1.2	3.4	3.4	0	0.06	0
None-poly(vinyl chloride) resin	granular	1.0	1.0	1.8	1.8	0.6	1.9	1.9	4.3	0.03	0.08	0

* Denotes material utilized by marine bacteria.
† Denotes figure used to determine Index of Oxygen Consumption in cases of exception to the rule.

TABLE II — CONTINUED

Material tested	Form of material tested	Individual 20° BOD test results — per cent available oxygen consumed				Individual 5° BOD test results — per cent available oxygen consumed				Index of oxygen consumption — per cent per day		Anaerobic test results — total H_2S produced
		1 week	2 weeks	4 weeks	8 weeks	1 week	2 weeks	4 weeks	8 weeks	20°C	5°C	16 weeks
Polyethylene												
0.2 melt index + antioxidant	sheet	5.4	10.8	10.8	10.8	0	0	0	0	0.2	0	0
0.2 melt index + antioxidant + 5% butyl rubber	sheet	2.4	4.4	5.5	9.9	0	0	0	0	0.2	0	0.7
0.2 melt index (Source B)	sheet	1.9	6.9	7.6	7.6	1.2	2.0	2.0	3.4	0.1	0.06	0.3
2.0 melt index — standard molecular weight polyethylene	granular	0	0	0	0					0		0
0.2 melt index (Source A) high molecular weight polyethylene	granular	0	0	0	6.4	0	0.8	0.8	0.8	0.1	0.01	0
0.2 melt index (Source B)	granular	0	0	0	2.7					0.05		0
0.2 melt index + antioxidant	granular	0	0	0	0					0		0
0.2 melt index + antioxidant + 5% butyl rubber	granular	0	0	0						0		0
0.7 melt index (high density + antioxidant), natural	sheet	0	0	0	0.8	0	0	0	0	0.01	0	0
0.7 melt index (high density), black	sheet	1.8	1.8	1.8	1.8	0	0	0	0	0.03	0	0

Polypropylene												
Polypropylene + antioxidant A	sheet	2.2	2.3	3.3	3.3	0	0	0.7	2.9	0.06	0.05	0
Polypropylene + antioxidant B	sheet	3.2	5.7	11.7	18.4	0.4	1.1	2.1	3.2	0.3	0.06	1.3
Other thermoplastics												
Cellulose acetate	film	2.0	2.0	4.0	6.7	0	0	0.7	13.6	0.1	0.2	0
Cellulose triacetate	film	4.9	4.9	4.9	4.9	0	0	3.3	3.3	0.09	0.06	0
6-6 polyamide (Source Ia)	film	0	1.6	4.0	8.4	0.4	1.1	1.1	2.2	0.2	0.04	0
6 polyamide (Source II)	film	4.2	4.2	4.4	4.4	0	2.2	2.2	2.2	0.08	0.04	0
6-10 polyamide (Source III)	film	1.1	1.1	1.1	1.5	0.3	0.3	0.3	1.4	0.03	0.03	0
Polycarbonate	film	2.7	2.7	3.2	4.3	0	1.4	1.4	1.4	0.08	0.03	0
Polyether	film	0.7	0.7	2.0	2.3	0	0.4	1.4	1.4	0.04	0.03	0
Polyethylene terephthalate	film	0.4	1.6	1.6	1.6	0	0	0	0	0.03	0	0
Polytetrafluoroethylene	sheet	1.5	1.5	1.5	1.5	0	0.8	0.8	1.6	0.03	0.03	0
Trithene film	film	0.3	0.3	0.8	0.8	0	0	0	1.8	0.01	0.03	0
Fibers												
Jute, untreated roving	fiber	18.5	(17 days) 56.6	45.0	100	1.6	7.3	9.4	54.2	2.0*	1.0*	51.6*
Acrylonitrile-vinyl chloride copolymer (washed)	fiber	0	0	0.7	0.7	0	0	0	0.4	0.01	0.01	0.5

* Denotes material utilized by marine bacteria.

TABLE II—CONTINUED

Material tested	Form of material tested	Individual 20° BOD test results — per cent available oxygen consumed				Individual 5° BOD test results — per cent available oxygen consumed				Index of oxygen consumption — per cent per day		Anaerobic test results — total H_2S produced
		1 week	2 weeks	4 weeks	8 weeks	1 week	2 weeks	4 weeks	8 weeks	20°C	5°C	16 weeks
Fibers — Continued												
Acrylonitrile-vinyl chloride copolymer (unwashed)	fiber					0	0	0	1.9		0.03	0.5
6-6 polyamide (Source Ib) (washed)	fiber	1.1	1.1	1.1	1.5	0	0	0	0	0.03	0	0.2
6-6 polyamide (Source Ib) (unwashed)	fiber	0	0	0	3.5	0	0	0	0	0.06	0	0
Casting compounds												
Styrene-polyester, silica filled	sheet	4.5	4.5	9.1	22.5	0	0	1.6	2.0	0.4	0.04	0.6
Styrene-polyester, silica filled	granular	13.2	14.3	14.3	25.0					0.4		0
Epoxide casting compound	granular	3.8	5.7	5.7	6.6					0.1		0
Epoxide casting compound	sheet	0	0	0.1	2.0	0	0	0.2	2.5	0.04	0.04	0

TABLE III—COMPARISON OF 20° AND 5°C BOD TEST RESULTS BASED ON INDEX OF OXYGEN CONSUMPTION

Material tested	Per cent oxygen consumed per day	
	20°C test	5°C test
Elastomers		
GR-S jacket Natural rubber jacket Neoprene red lead jacket (with clay) Neoprene red lead jacket (without clay) Natural rubber insulation GR-S insulation	10–20%	1–2%
GR-A jacket Neoprene (ZnO, MgO jacket)	under 10%	under 1%
Butyl rubber jacket	10–20%	under 1%
Silicone rubber	under 10%	1–2%
Jute		
Untreated roving	2%	1%
Poly(vinyl chloride), designated by plasticizer		
Polyester E Polyester A	over 10%	over 2%
Di-2-ethylhexyl phthalate (DOP) — Source 1 None (rigid)	2.5–10%	0.5–2%
Tri-2-ethylhexyl phosphate (TOP) Nitrile rubber/polyester C Nitrile rubber Tricresyl phosphate (TCP) Di-2-ethylhexyl phthalate (DOP) — Source 3 Proprietary compound Di-2-ethylhexyl phthalate (DOP) — Source 2	0.5–2.5%	0.1–0.5%

Examination of the individual curves containing the BOD data for the externally plasticized poly(vinyl chloride) plastics shows some similarity to the rates of attack found in the rubber samples. At 20°C nine different test materials were utilized essentially in a manner which would fall into the straight-line or Type A oxygen-consumption rate curve. The two exceptions were the rigid unplasticized poly(vinyl chloride), which began to be utilized more slowly as incubation time increased, and the poly(vinyl chloride) plasticized with tricresyl phosphate, which began to be attacked more rapidly as time increased. At the 5°C incubation temperature only the poly(vinyl chloride) plasticized with poly-

TABLE IV—MATERIALS NOT ATTACKED IN EITHER BOD OR ANAEROBIC TESTS

Poly(vinyl chloride)
Semiflexible copolymer A (no plasticizer added)
Semiflexible copolymer B (no plasticizer added)
Pure poly(vinyl chloride) resin
Polyethylene
2.0 melt index, standard molecular weight
0.2 melt index (Source A), high molecular weight
0.2 melt index (Source B), high molecular weight
0.2 melt index + antioxidant
0.2 melt index + 5% butyl rubber + antioxidant
0.7 melt index (high density) + antioxidant
0.7 melt index (high density) + carbon black + antioxidant
Polypropylene
Polypropylene + antioxidant A
Polypropylene + antioxidant B
Other thermoplastics
Polyamide (Sources Ia, II, and III)
Polyethylene terephthalate
Trithene film
Polytetrafluoroethylene
Polyether
Cellulose triacetate
Cellulose acetate
Polycarbonate
Casting compounds
Epoxide casting compound
Styrene-polyester, silica filled
Fibers
Acrylonitrile-vinyl chloride copolymer
6-6 polyamide (Source Ih)

ester E was utilized following the Type A pattern, while five samples — the rigid unplasticized poly(vinyl chloride) and those plasticized with polyester A, nitrile rubber, DOP Source 3, and a proprietary plasticizer — were attacked more slowly with an increase in incubation time. The five other poly(vinyl chloride) plasticizers — DOP Source 1, TOP, nitrile rubber/polyester C, TCP, and DOP Source 2 — were more easily attacked as incubation time increased.

It is highly possible that the slope variations noted in the individual

Type B curves could have resulted from testing under laboratory conditions. For instance, when a material has been attacked quite slowly at the beginning of a test and then more rapidly as the test progresses, it may be due to the fact that an equilibrium had to be established within the confines of the 60-milliliter test bottle before the bacteria could function effectively against the test material. Or perhaps the bacteria were unable to attack until a leachable plasticizer or lubricant or other compounding material had been released into the sea water in a form or amount that would be acceptable to the bacteria. Another factor which could be partially responsible for this type of situation was the fact that the enrichment cultures which were used for the 5°C tests were originally incubated at 25°C; this temperature could have temporarily inactivated bacteria, allowing them to become active only after incubation at 5°C.

With the Type C curve, laboratory conditions could also be held partially responsible for its shape. Possible explanations for a greater rate of attack at the beginning rather than at the end of the test might be that toxic materials were being released into the sea water, thus continually diminishing the rate of attack, or perhaps that the establishment of an equilibrium brought about a dormant system causing a decrease in microbial activity. This suggests that attack of the same materials in a natural marine exposure might follow a different pattern.

In comparing the curves for the same materials under different incubation temperatures it was noted that the only materials which showed similar patterns of attack at both temperatures were the poly(vinyl chloride) samples plasticized with polyester E and TCP, the unplasticized rigid poly(vinyl chloride), and the elastomer neoprene (containing zinc oxide and magnesium oxide) jacket. In all cases the rate of utilization was noticeably higher during the 20°C test.

In the remainder of the testing of rubber compounds the greatest difference between the patterns of oxygen consumption at 20° and 5° was noted during the first two weeks of incubation. The temperature differential appeared to be responsible for the length of incubation time needed to set up an active microbial system. This situation was also encountered in the testing of the four poly(vinyl chloride) samples plasticized with DOP Source 1, TOP, nitrile rubber/polyester C and DOP Source 2 at 5°C, in which the oxygen consumption rate increased as incubation time increased. These same materials followed the Type A pattern of oxygen depletion when incubated at 20°C.

The remaining four externally plasticized poly(vinyl chloride) plastics were attacked essentially in direct proportion to the extent of time in

test when incubated at 20°C, but exhibited a higher rate of attack at the beginning than at the end when tested at 5°C.

One other interesting feature was noted when comparing the curves of the individual materials at the two incubation temperatures. In a few cases, such as with the DOP Source 1 plasticized poly(vinyl chloride) sample, there was a short interval in both curves where the slope had leveled off. In the cited example this interval represented the second week of the 20°C test and the third and fourth week of the test, when the temperature was held at 5°C.

In comparing the indices of oxygen consumption of the individual materials at the two temperatures, the most noticeable feature appears to be that the elastomeric materials, when incubated at 20°C, were utilized essentially ten times faster than the same materials incubated at 5°C. Furthermore, examination of the relationship between BOD tests at 20° and 5°C involving the externally plasticized poly(vinyl chloride) samples shows that the ratio of attack was approximately 5 to 1. The ratio of the rates of attack witnessed in the BOD testing of jute was 2 to 1. No other ratios were noted for less utilized material, since the indices for both 20° and 5° data were too small to allow any accurate comparisions.

This classification system does not necessarily mean that the test data for each material exhibit this type of ratio. For instance, the relationship of attack at the two temperatures for polyester E plasticized poly(vinyl chloride) would be 14.3 to 5.1, for a ratio of 2.8 to 1 instead of 5 to 1, and for natural rubber insulation it would be 7.8 to 1 instead of 10 to 1. One extreme case was the BOD results for neoprene (containing zinc oxide and magnesium oxide) jacket, which gave the material an index of 2.8 per cent per day and a place on the "under 10 per cent" for 20°C data, while the 5°C index of 0.1 per cent per day correctly placed the material on the related "under 1 per cent" list. However, this produced an actual 28.0 to 1 ratio in the rates of attack. This is a broad classification of the performance ratios and, although most of the materials fall essentially within the assumed ratios, it is not infallible.

The materials which failed to fall into this group relationship were the elastomers butyl jacket and silicone rubber. The ratio of the results of the silicone rubber BOD tests were 9.7 to 1.4, thus being fairly close to the range of anticipated behavior. The placement difficulty came from the choice of 10.0 rather than 9.0 or even 9.7 as a breaking point between the two groups, and does not indicate any peculiarity of sample performance. In the case of butyl rubber jacket, the BOD tests have been rerun in an effort to determine its odd behavior when compared to the

remainder of the elastomers. However, in all tests conducted to date butyl jacket has not been readily attacked at 5°C. The reason for this phenomenon is not fully understood.

In studying these figures it may be noted that the elastomers were utilized by aerobic bacteria more readily than was any other group of materials. The total available oxygen was consumed in all of the ten BOD tests run on elastomers at 20°C. The time required for complete consumption ranged from 7 to 28 days with the indices of oxygen consumption varying from 19.9 to 2.8 per cent per day. In the 5°C tests the elastomers were usually attacked but at a slower rate, and butyl jacket, GR-A jacket and neoprene jacket containing zinc oxide and magnesium oxide still showed some resistance after eight weeks of incubation. Only three of the elastomers, namely butyl and natural rubber jackets and natural rubber insulation, served as a source of energy for the anaerobic bacteria.

The remaining general class of materials which provided a food source for the marine bacteria was the externally plasticized poly(vinyl chloride) samples. In all poly(vinyl chloride) tests that showed high oxygen consumption the materials in question had been compounded with fatty-acid-type lubricants or external plasticizers that contained fatty acids. Three of the group which were studied and showed no attack were the pure resin and two internally plasticized semiflexible copolymers. These observations indicate that the plasticizer or lubricant and not the poly(vinyl chloride) itself was utilized as a source of carbon. With incubation at 5°C the rate of attack was much lower, but the samples plasticized with polyesters E and A, fatty acid compounds, were attacked considerably faster at both temperatures than the remaining poly(vinyl chloride) samples. Also, these samples were readily attacked by anaerobes during 16 weeks of incubation.

In all tests conducted jute fiber was readily utilized, but it is rather difficult to compare rates of attack on jute with the various sheet materials due to the great variation in material surface area made available to the bacteria. The performances of cellulose acetate at 5°C and styrene-polyester and polypropylene containing antioxidant B at 20° were close to the arbitrary 0.5 per cent per day consumption index, but cannot be reported as definitely showing attack.

The majority of the anaerobic test data available was reported previously by Snoke[3] but are also presented here in Table II in order to include all the laboratory findings in a single document. In general, only materials that were attacked by aerobic organisms have tended to show attack by anaerobic sulfate-reducing bacteria, and usually this was to a

lesser degree and over a longer period of incubation. Since the rate of attack was usually very slow, the anaerobic tests were only run at 20°, where, it was assumed, the higher hydrogen sulfide production would be. There were three cases of doubtful performances in this group, natural rubber insulation, poly(vinyl chloride) DOP Source 1 plasticizer, and the polypropylene sample containing antioxidant B.

VII. SUMMARY

The following general statements are made regarding the relationship between the 5° and 20°C BOD tests run on 57 materials to date:

1. Oxygen was consumed significantly during incubation at both temperatures for all the externally plasticized poly(vinyl chloride) samples and for all the elastomeric materials except neoprene jacket containing zinc oxide and magnesium oxide when incubated at 5°C. Jute was also utilized at both temperatures.

2. In each of the above exceptions the microbial activity, measured by the oxygen consumed, was greater during the 20°C incubation tests. The ratio of the rates of attack at the two temperatures for the elastomers was generally 10 to 1, while it was 5 to 1 for the externally plasticized poly(vinyl chloride) samples and 2 to 1 for the untreated jute roving.

3. No one figure could be used to describe adequately the rate of attack in any test, since the rates appeared to vary during the several intermediate incubation intervals. Sometimes the rate of attack seemed to be in direct proportion to the length of time of incubation, but quite often the rates either decreased or increased with an increase in incubation time. The type of rate curve for the same material at the two temperatures was often dissimilar.

4. One number was selected for each material representing an assumed average oxygen consumption per day. This figure, designated the Index of Oxygen Consumption, is presented as a means of simplifying the presentation of the data obtained from each test.

5. The oxygen consumption with three materials — cellulose acetate when incubated at 5°C and silica-filled styrene-polyester and polypropylene containing antioxidant B at 20°C — although very low, was somewhat higher than comparable control values.

6. The following were not attacked at either temperature:

   a. Polyethylene

   b. Internally plasticized poly(vinyl chloride) copolymers and pure poly(vinyl chloride) resin

e. Other thermoplasties with the possible exeeption of cellulose aeetate at 5°C

d. Epoxide easting compound

e. Synthetie fibers acrylonitrile-vinyl chloride copolymer and 6-6 polyamide (Source Ib).

Although incubated for a longer time, the samples exposed to anaerobic conditions showed less utilization by sulfate-reducing bacteria. Only three elastomers, six externally plasticized poly(vinyl chloride) samples, and jute showed noticeable attack following 16 weeks incubation under anaerobic conditions at 20°C.

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REFERENCES

1. ZoBell, C. E., and Beckwith, J., The Deterioration of Rubber Products by Microorganisms, J. Amer. Water Works Assoc., **36**, 1944, p. 439.
2. ZoBell, C. E., Action of Microorganisms on Hydrocarbons, Bact. Rev., **10**, 1946, nos. 1–2.
3. Snoke, L. R., Resistance of Materials to Marine Biological Attack, B.S.T.J., **36**, 1957, p. 1095.
4. ZoBell, C. E., *Marine Microbiology*, Chronica Botanica Co., Waltham, Mass., 1944.
5. ZoBell, C. E., Bacteria of the Marine World, Sci. Monthly, **55**, 1942, p. 320.
6. Hesse, E., Bakteriologische Untersuchungen auf einer Fahrt nach Island Spitzbergen und Norwegen im Juli 1913, Centralbl. für Bakt., **72**, 1914, p. 454.
7. Waksman, S. A., and Carey, C. L., Decomposition of Organic Matter in Sea Water by Bacteria. II. Influence of Addition of Organic Substances upon Bacterial Activities, J. Bact., **29**, 1935, p. 545.
8. Morita, R. Y., and ZoBell, C. E., Bacteria in Marine Sediments, Office of Naval Research, Research Reviews, July 1956, p. 21.
9. Cattell, M., The Physiological Effects of Pressure, Biol. Rev., **11**, 1936, p. 441.
10. Sanborn, J. R., Certain Relationships of Marine Bacteria to the Decomposition of Fish, J. Bact., **19**, 1930, p. 375.